Romer Labs
AgraStrip Quantitative Total Fumonisin (FUM) Test

TABLE OF CONTENTS **PAGE**

GENERAL INFORMATION

The AgraStrip Quantitative Total Fumonisin (FUM) Test is a one-step lateral flow immunochromatographic assay for the quantitative screening of total fumonisins (FB1, FB2 and FB3) in samples. The test is based on a competition immunoassay format. Antibody-particle complex (conjugate) coated in a microwell is dissolved in sample extract and mixed with assay buffer. A FUM strip is placed into the microwell. The mixed content is then wicked onto a membrane of the FUM strip, which contains a test zone and a control zone. The test zone captures free antibody-particle complex (conjugate), allowing color particles to concentrate and form a visible line. The color intensity of the line is inversely proportional to the concentration of FUM in the sample. The line is always visible in the control zone regardless of the presence of FUM. The FUM strips are then measured using an AgraVision Reader and the results are determined.

The instructions presented in this document cover only the procedure for performing the analytical test for official inspections. For questions regarding this procedure, contact Dr. Ajit Ghosh of the Technology and Science Division by phone at 816-891-0417 or email at Ajit.K.Ghosh@usda.gov.

Refer to the current policies and/or instructions issued by the Policies, Procedures, and Market Analysis Branch (PPMAB) of the Field Management Division for information on use of this test kit in official inspections including sampling, general sample preparation (e.g., grinding and dividing), reporting and certification of test results, laboratory safety, and hazardous waste management. For questions regarding these policies and/or instructions, contact Patrick McCluskey of PPMAB by phone at 816-659-8403 or email at Patrick.J.McCluskey@udsa.gov.

Approved Test Kit Information

Test Kit Vendor:	*Romer Labs, Inc. (636) 583-8600*
Test Kit Name:	AgraStrip Quantitative Total Fumonisin (FUM) Test
Product Number:	COKAS3000A
Effective Date of Instructions:	06/08/2015
Instructions Revision Number	0
Conformance Range:	0.5 – 5.0 ppm
Number of Analyses to Cover Conformance Range:	1
Type of Service:	Quantitative
Supplemental Analysis:	Yes
Approved Commodities:	Corn, corn gluten meal, distiller's dried grains with solubles (DDGS), and wheat
Extraction method:	

	Shake 50-gram sample with 100 milliliters (mL) of 70% methanol/30% deionized or distilled water (v/v) by hand for 1 minute.
Test Format:	Lateral Flow Strip
Detection Method:	AgraVision Reader (Product Model No. EQASR1000)

PREPARATION OF TESTING MATERIALS

All reagents and kit components must be at room temperature 20-24°C (68-75°F) before use. The temperature of AgraStrip Incubator is set at 35°C.

1. Place Assay Buffer bottle in the AgraStrip heat block in the AgraStrip Incubator and incubate at 35°C for 30 minutes. During shipment the Assay Buffer will precipitate and during this 30 minutes heat treatment it will completely re-dissolve. After the 30 minutes incubation, shake the Assay Buffer bottle to properly mix its contents to be homogeneous.

Note: It is recommended to switch on the incubator in the morning and to keep it on throughout the whole day.

Preparation of Extraction Solvent: 70% MeOH/30% deionized or distilled water (v/v):

Note: 70/30 may be purchased pre-mixed. One can also prepared by following the procedure below.

(1) Using a 1000 mL graduated cylinder, measure 700 mL of methanol (ACS grade) and place it into a clean carboy with spigot.

(2) Using a 500 mL graduated cylinder, measure 300 mL deionized or distilled water and add into the methanol and shake until it is completely mixed.

(3) Label the container stating the mixture (70 percent methanol and 30 percent water), date of preparation, and initials of technician who prepared the solution.

(4) Store this solution at room temperature in a tightly closed container until needed (maximum 12 months).

SAMPLE PREPERATION AND EXTRACTION PROCEDURES

Standard Extraction Procedure for corn, corn gluten meal, DDGS, and wheat

(1) Weigh out 50 grams (+/- 0.2 g) of ground sample into the Whirl-Pak bag.

(2) Add 100 mL of extraction solvent and securely close the Whirl-Pak bag.

(3) Vigorously shake the closed Whirl-Pak bag by hand for 1 minute.

(4) Allow sample to settle to get particle free supernatant for 2.5 minutes.

(5) The supernatant of the sample extract is ready for dilution procedure.

Note: The extract should be used immediately after the 2.5 min settling time is complete. If it is to be kept, the supernatant should be separated from the sample.

Dilution Procedure for corn

(1) Dilute the sample extract 1:40 with dilution buffer. Prepare dilution immediately before analysis.

(2) Pipette 2000 µL of Dilution buffer into a dilution tube. Then, use a yellow or white pipette tip to remove and discard 50 µL of that buffer.

(3) Pipette 50 µL of extract (without particle free) into the dilution tube containing 1950 µL dilution buffer.

(4) Mix well by inverting the tube 5 times and it is ready for the analysis.

Dilution Procedure for wheat

(1) Dilute the sample extract 1:20 with dilution buffer. Prepare dilution immediately before analysis.

(2) Pipette 1000 µL of Dilution buffer into a dilution tube. Then, use a yellow or white pipette tip to remove and discard 50 µL of that buffer.

(3) Pipette 50 µL of extract (without particle free) into the dilution tube containing 950 µL dilution buffer.

(4) Mix well by inverting the tube 5 times and it is ready for the analysis

Dilution Procedure for corn gluten meal, DDGS

(1) Dilute the sample extract 1:25 with dilution buffer. Prepare dilution immediately before analysis.

(2) Pipette 1250 µL of Dilution buffer into a dilution tube. Then, use a yellow or white pipette tip to remove and discard 50 µL of that buffer.

(3) Pipette 50 µL of extract (without particle free) into the dilution tube containing 1200 µL dilution buffer.

(4) Mix well by inverting the tube 5 times and it is ready for the analysis

TEST PROCEDURES

a. Analysis Procedure

(1) Place the cover of the heat block on the top of the heat block. Break off one conjugate coated microwells for one sample to be run. Remove sealing tape from conjugate microwell, and place the conjugate microwell inside the heat block. Ensure that the well is fully seated in the heat block. Only one sample should be run at one time.

(2) Add 50 µL of diluted sample extract to the conjugate microwell.

(3) Place the cover on the heat block to cover the microwell and incubate for 30 seconds.

(4) Lift up the cover and immediately add 50 µL of assay buffer into the microwell.

(5) Mix the content in the microwell by **pipetting it up and down 10 times**.

(6) Put one test strip into one microwell. Place the cover back into the heat block to cover the microwell and the test strip.

(7) Allow the test strip to develop color for 3 minutes.

(8) Wipe the end of the strip test onto an absorbent paper and insert the strip into the strip holder for reading.

b. Reading the Results

(1) Use the AgraVision Reader to read the strip and interpret result. **Note:** follow the instructions of the AgraVision Reader to read the strips. Strips should not be read more than 1 minute after completion of 3 minutes development.

(2) Turn on the AgraVision reader using the power button located on the back.

(3) Use the arrow keys on the keypad to highlight “TEST”. Select it using the checkmark key.

(4) Use the arrow keys on the keypad to highlight “Mycotoxin”. Select it using the checkmark key.

(5) The barcode scanner will turn on. Scan the barcode, found on the microwell tube included in the test kit.

(6) Create a sample ID by using the alphanumeric keys on the keypad. Use the checkmark key to enter.

(7) If a second strip is to be read at the same time, repeat steps 11 and 12 for slot 2. If only one strip is being read, use the pound key to bypass to the next screen.

(8) Enter the operator ID. Press the checkmark key to enter, and press it a second time to move to the next screen.

(9) The reader is ready to read and will display "start measurement". Insert the strips into the tray, and insert the tray into the reader. The strips should go in the tray with the white end facing into the reader. Press the checkmark key to read.

(10) After completion of reading, press the checkmark key to save the result in the AgraVision Reader's memory, or the pound key to print the result.

Note: after the test, the used microwells can be removed easily with a tweezers provided with the kit.

c. Interpretation of Results

(1) A color line always appears in the upper section of the test strip to indicate that the test strip is working properly. This line is the Control Line (C). A line in the lower section of the test strip indicates the test result. This line is the Test Line (T).

(2) **Invalid results:** If there is no control line in the control zone, the test is invalid and the sample should be re-tested by using a new test strip.

(3) **Valid results:** 2 lines are visible. The intensity of the line in the test zone is dependent on fumonisin concentration in the sample and must be measured with an AgraVision Reader.

REPORTING AND CERTIFYING TEST RESULTS

Refer to the current instructions issued by the Policies, Procedures, and Market Analysis Branch of the Field Management Division for reporting and certification of test results. For questions regarding these instructions, contact Patrick McCluskey (816-659-8403 or Patrick.J.McCluskey@udsa.gov).

SUPPLEMENTAL ANALYSIS

Supplemental analysis (corn only) is a procedure followed when a result is observed above the upper limit of the concentration range used in GIPSA's test kit performance evaluation.

The range for performance evaluation of quantitative fumonisin test kits is 0.5 – 5 ppm. Therefore, supplemental analysis would be performed for a result above 5 ppm. In supplemental analysis, the extract is diluted with a higher dilution factor so the resulting concentration is between the lower and upper limits of the test kit evaluation range (i.e., 0.5 – 5 ppm for fumonisin), and a correction for dilution is applied to derive at the final result. For this test kit, the appropriate calibration setting is selected for automatic correction for the supplemental dilution performed. Supplemental analysis is performed only at the request of the applicant.

a. For supplemental analysis, perform the following procedure

(1) Follow the above "**Test Procedure**" on page 4 with these 2 exceptions:

(2) Dilute the sample extract with extraction solvent with dilution factor of 80X (25uL extract 1975uL Dilution buffer). Then proceed to the test procedure described above.

(3) Calculate the result by multiplying the reading from the AgraVision Reader with the dilution factor 2.

A final result less than 3.5 ppm is indicative of a problem, and troubleshooting is needed. Verify the procedure is being followed properly. Perform the procedure for the Diluted Extract (non-supplemental analysis) and only perform the supplemental analysis again if the value is greater than 5.0 ppm.

STORAGE CONDITIONS AND PRECAUTIONS:

a. Storage Conditions

(1) Store test kits at 2-8°C (35-46°F) when not in use, and do not use beyond the expiration date. Do not freeze. Do not leave it in direct sunlight.

(2) Test strips must be kept inside their original tubes.

(3) Conjugate microwells must be kept inside their original tubes.

b. Precautions

(1) All reagents must be at room temperature before the assay is running.

(2) Adhere to the instructions of test procedures.

(3) Do not re-use test strips and conjugate wells.

(4) Consider all materials, containers and devices that are exposed to the sample to be contaminated with toxin. Wear protective gloves and safety glasses when using the kit.

(5) The components in this test kit have been quality control tested as a standard batch unit. Do not mix components from different lot numbers.

EQUIPMENT AND SUPPLIES:

a. Materials Supplied with the Kit:

(1) 1 tube containing 24 AgraStrip Fumonisin test strips

(2) 1 tube containing 24 AgraStrip Fumonisin Conjugate wells with lyophilized antibody particle complex (conjugate)

(3) 1 bottle of 50 mL AgraStrip Fumonisin Dilution Buffer

(4) 1 bottle of 1.5mL of AgraStrip Fumonisin Assay Buffer

(5) 1 bag of 72 yellow or white pipette tips; 1 bag of 24 blue pipette tips

(6) 1 bag of 24 microcentrifuge tubes (dilution tubes); 24 Whirl-Pak bags

b. Materials Required but not Provided with Kit

Extraction Procedure

(1) Romer Series II Mill or equivalent

(2) EQOLE1010: Balance, 400 grams

(3) EQOLE1050: Graduated cylinder: to hold 100 mL

(4) 70/30 Methanol/Water

Assay Procedure

(1) Single channel pipette capable of pipetting up to 100 μL.

(2) Single channel pipette capable of pipetting up to 1000 μL.

(3) EQOLE1300: Timer

(4) EQASR1003:AgraVision Reader without printer or EQASR1000: AgraVision Reader with printer

(5) EQOEV2060: AgraStrip Incubator with timer or EQASR1005: AgraStrip heat block with cover.

REVISION HISTORY

Revision 0 (06/08/2015)